A Research Note

EVALUATION OF ASCORBYL MONOESTERS FOR THE INHIBITION OF NITROSOPYRROLIDINE FORMATION IN A MODEL SYSTEM

ABSTRACT

The effect of a number of ascorbic acid derivatives on the nitrosation of pyrrolidine was determined in a model system developed to simulate the lipid-aqueous-protein composition of bacon. Sodium ascorbate reduced nitrosopyrrolidine formation 43% in the aqueous phase, but had little effect in the lipid layer. The combination of several ascorbyl esters with sodium ascorbate increased the inhibitory effect to a high of 70% in the aqueous phase. In the lipid phase up to 49% reduction in nitrosation was observed.

INTRODUCTION

ASCORBIC ACID (Vitamin C), its isomer erythorbic acid and their salts have been permitted in cured meat products since 1955 (USDA Memorandum No. 217, June 1955). Initially, they were used to accelerate the formation of the stable pink-red color (nitrosyl hemochrome) of cured meats. Recently however, sodium ascorbate (NaAsc) and sodium erythorbate (NaEry) were found to inhibit nitrosamine formation in frankfurters (Fiddler et al., 1973) and bacon prepared with higher than permitted levels of NaAsc (Herring, 1973). It is postulated that NaAsc and NaEry inhibit nitrosamine formation because the rate of reaction of nitrite with the reductants is greater than the rate of nitrosation of the secondary amines, depending on their basicity. However, they do not completely prevent nitrosamine formation in bacon.

We have shown (Fiddler et al., 1974) that nitrosopyrrolidine (NO-Pyr) is formed primarily in the adipose tissue of bacon. The nitrite reducing ability of the reductants in adipose tissue may be limited by their fat solubility. Therefore, experiments with more fat soluble derivatives of ascorbic acid, in combination with NaAsc, were undertaken to determine whether a more effective means of inhibiting the nitrosation of pyrrolidine could be obtained in a model system simulating the composition of bacon. The results of these experiments are reported herein.

EXPERIMENTAL

ASCORBYL PALMITATE, ascorbyl laurate, erythorbyl palmitate, erythorbyl laurate and the potassium salt of ascorbyl-2-sulfate were obtained from Dr. Winifred Cort, Hoffmann-LaRoche. These ascorbyl monoesters were prepared by known procedures and their identities have been established. In addition, these compounds were purified by chromatographic methods (by the suppliers) to a purity in excess of 99%. The magnesium salt of ascorbyl-2-phosphate was obtained through the courtesy of Dr. Paul Seib, Kansas State University (Seib et al., 1974). Ascorbyl oleate was prepared by Dr. Leonard Silbert, Animal Fat Products Laboratory, Eastern Regional Research Center. Pyrrolidine, sodium nitrite, and sodium ascorbate were obtained from commercial suppliers.

The study was carried out in a model system consisting of 60g of safflower oil, 30g of pH 6.00 aqueous buffer (1N NaOH, 1M KH_2PO_4), 8.0g bovine albumin, 1.5g sodium chloride and 0.5g sodium tripolyphosphate. This would correspond to bacon having a composition of 60% fat, 30% H_2O, 8% protein and 2% ash. To this system was added 1×10^{-4} mole of pyrrolidine (71 ppm), 2.52×10^{-4} mole of sodium ascorbate (5.04×10^{-4} mole in the control reaction), 2.52×10^{-4} mole of the compound to be tested, and 2.17×10^{-4} mole of sodium nitrite (150 ppm). The mixtures were stirred and heated at 52°C for 2 hr. The reaction mixtures were cooled to room temperature, then centrifuged at 6000 rpm for 10 min to facilitate separation of the oil and aqueous layers with a separatory funnel. To precipitate the protein, 40 ml of 25% trichloroacetic acid (TCA) was added to the aqueous layer (A) and 10 ml of 25% TCA to the fat layer (B). After centrifuging at 6000 rpm for 5 min, the aqueous layer (A) was decanted into a separatory funnel containing 10 ml of 5N NaOH, mixed, then extracted three times with 100 ml of methylene chloride (CH_2Cl_2). The combined CH_2Cl_2 extracts were washed with 50 ml of 6N HCl, then 50 ml of 5N NaOH and separated. The CH_2Cl_2 extract was dried by passing it through anhydrous sodium sulfate into a Kuderna-Danish apparatus and concentrated to 4 ml in a steam bath prior to gas chromatographic analysis. The remaining oil layer (B) was decanted into a separatory funnel containing 5 ml of 5N NaOH using 60 ml of n-hexane to facilitate the transfer. This mixture was extracted three times with 50 ml of water, the water washes combined, extracted with CH_2Cl_2 and treated in the same manner as described for the aqueous layer (A). An average total recovery of 79% was obtained using a sample to which 5 ppb NO-Pyr had been added and carried through the entire procedure.

The NO-Pyr was determined with a Varian Aerograph model 1740-1 gas chromatograph equipped with an alkali flame ionization detector as described previously (Howard et al., 1970). A 9-1/2 ft × 1/8 in. stainless steel column packed with 15% Carbowax 20M-TPA on 60–80 mesh Gas Chrom P was used with the following operating conditions: column temperature, 170°C; injector port, 185°C; detector, 225°C. The flow rates were: helium-60, air-215, and hydrogen-86 ml/min. Conditions for confirmation of NO-Pyr by GLC-high resolution mass spectrometry have been reported previously (Pensabene et al., 1974).

RESULTS & DISCUSSION

THE EFFECTS of some ascorbate derivatives on the nitrosation of pyrrolidine in a lipid-aqueous-protein system are shown in Table 1. The amount of nitrosopyrrolidine in the lipid layer was considerably less than that in the aqueous-protein portion contrary to reported findings in bacon (Fiddler et al., 1974). This may reflect the greater solubility of pyrrolidine, and its nitrosated form, in the aqueous phase and the possibility that the nitrosopyrrolidine was not forced into the lipid layer by evaporation of the water, which could occur during the frying of bacon. Alternatively, NO-Pyr in the lipid phase may be higher due to the high temperature achieved during frying, which increases the rate of nitrosation. The results, therefore, demonstrate the overall effect of the esters on nitrosation in both the aqueous and lipid phases of the system. Sodium ascorbate at 500 and 1000 ppm inhibited NO-Pyr formation in the aqueous phase 43.0 and 85.5%, respectively. No interference with NO-Pyr formation occurred in the lipid phase. However, this finding cannot be explained at this time. The esters

Table 1—Ascorbyl monoesters as inhibitors of the nitrosation of pyrrolidine in a model system

Compound[a]	Ascorbyl[b] ester (ppm)	Nitrosopyrrolidine[c,d] Aqueous ppb	Aqueous % Reduction	Lipid ppb	Lipid % Reduction
None	–	167	–	10.5	–
NaAsc (1000 ppm)	–	24.2	85.5	11.4	(+ 8.6)
NaAsc (500 ppm)	–	95.2	43.0	14.8	(+41.0)
NaAsc (500 ppm) + Asc palmitate	1046	63.5	62.0	5.4	48.6
Asc laurate	905	72.6	56.5	6.3	40.0
Asc oleate	1110	62.4	62.6	6.6	37.2
Ery palmitate	1046	56.3	66.3	6.8	35.2
Ery laurate	905	123	26.3	7.7	26.7
K_2 Asc-2-sulfate	880	50.4	69.8	5.8	44.8
Mg Asc-2-phosphate	731	82.4	50.7	9.5	9.5

[a] Plus pyrrolidine and $NaNO_2$
[b] Equimolar with NaAsc, 2.52×10^{-4} mole
[c] Confirmed by MS
[d] All values are the average of three experiments.

were tested in the presence of an equimolar concentration of NaAsc to determine whether additional inhibition of NO-Pyr formation occurred in a lipid system. The ascorbyl esters of oleic, palmitic and lauric acids had only a small effect on the nitrosation of pyrrolidine in the aqueous phase, increasing the inhibition due to the NaAsc alone only 13–20%. However, in the lipid phase, ascorbyl palmitate reduced nitrosation 48.6% while the other two esters inhibited the reaction about 40%. The erythorbyl ester of palmitic acid showed a slightly lower inhibitory effect in the lipid phase, reducing nitrosation by 35.2%, whereas erythorbyl laurate showed very little inhibition in either phase. The magnesium salt of ascorbyl-2-phosphate gave only a 9.5% reduction in the lipid phase and the potassium salt of ascorbyl-2-sulfate produced the same effect as the ascorbyl esters, reducing nitrosation by 44.8%. The later compound is of particular interest since it is 18 times more stable than potassium L-ascorbate (Quadri et al., 1973) in a boiling, aerated water system. Recently, Quadri et al. (1975) have shown that the potassium salt of ascorbyl-2-sulfate is generally more stable in bread, in pancake, in an extruded wheat product, and in whole pasteurized milk than L-ascorbate.

The results show that nitrosation can be reduced in both the aqueous and the lipid phases of the model system studied when NaAsc is used in combination with an ascorbyl ester. However, the esters, even though more soluble in the lipid phase than NaAsc, were only slightly fat soluble. It is possible, therefore, that a more fat soluble ascorbyl ester introduced into the adipose tissue might produce a greater reduction of nitrosamine, particularly in the preparation of cured meat products that contain a great deal of adipose tissue. Ascorbyl palmitate and the potassium salt of ascorbyl-2-sulfate are currently being investigated for their NO-Pyr inhibitory activity in bacon.

NOTE: Precaution should be exercised in the handling of nitrosamines since they are potential carcinogens.

REFERENCES

Fiddler, W., Pensabene, J.W., Fagan, J.C., Thorne, E.J., Piotrowski, E.G. and Wasserman, A.E. 1974. The role of lean and adipose tissue on the formation of nitrosopyrrolidine in fried bacon. J. Food Sci. 39: 1070.

Fiddler, W., Pensabene, J.W., Piotrowski, E.G., Doerr, R.C. and Wasserman, A.E. 1973. Use of sodium ascorbate or erythorbate to inhibit formation of N-nitrosodimethylamine in frankfurters. J. Food Sci. 38: 1084.

Herring, H.K. 1973. Effect of nitrite and other factors on the physicochemical characteristics and nitrosamine formation in bacon. Proceedings of the Meat Industry Research Conference, p. 47.

Howard, J.W., Fazio, T. and Watts, J.O. 1970. Extraction and gas chromatographic determination of N-nitrosodimethylamine in smoked fish: Application to smoked nitrite treated chub. J. Assoc. Offic. Anal. Chem. 53: 269.

Pensabene, J.W., Fiddler, W., Gates, R.A., Fagan, J.C. and Wasserman, A.E. 1974. Effect of frying and other cooking conditions on nitrosophyrrolidine formation in bacon. J. Food Sci. 39: 314.

Quadri, S.F., Seib, P.A. and Deyoe, C.W. 1973. Improved method of preparing L-ascorbic acid 2-sulfate. Carbohydrate Research 29: 259.

Quadri, S.F., Liang, Y.T., Seib, P.A., Deyoe, C.W. and Hoseney, R.C. 1975. Stability of L-ascorbate 2-sulfate and L-ascorbate in wheat foods and milk. J. Food Sci. 40: 837.

Seib, P.A., Liang, Y.T., Lee,C-H, Hoseney, R.C. and Deyoe, C.W. 1974. Synthesis and stability of L-ascorbate 2-sulfate, J. Chem. Soc. Perkin I: 1220.

USDA, Meat Inspection Branch. Use of ascorbic acid and sodium ascorbate in preparation of meat food products. Memorandum No. 217, June 15, 1955.

Ms received 7/2/75; revised 8/5/75; accepted 8/11/75.

Reference to brand or firm name does not constitute endorsement by the U.S. Dept. of Agriculture over others of a similar nature not mentioned.